CRL MASS SPECTROMETRY FACILITY

INSTRUMENT USER MANUAL

MALDI MICRO

Operating Instructions

Basement Spec Lab: 00.097

This is a guide to using the MALDI Micro for those who have received training. If you have any questions or problems whilst using this instrument please contact a member of the CRL Mass Spectrometry Facility Staff.

NOTE: ALL USERS MUST OBTAIN TRAINING FROM A MEMBER OF THE MASS SPECTROMETRY STAFF BEFORE USING THE MALDI MASS SPECTROMETER.

Introduction

The Waters MALDI micro MX can be operated in Linear or Reflectron modes. Linear covers the whole mass range of the instrument (0 – 500K Daltons) but has a low resolution (approximately 1000) whereas Reflectron gives much higher resolution (approximately 12,000) but the mass range is restricted (0 – 15K Daltons). Whilst both Positive and Negative ionisation can be used most sample types are run in positive ionisation. NOTE: The use of matrices in sample preparation usually raises the effective starting mass from 0 to around 500 Daltons.

Sample Preparation

The method used to prepare the sample, and the specific matrix to use will depend upon the sample characteristics. Chapter 6 (pp83 – 102) in the Operators Manual which is kept by the instrument, gives a description of the common matrices, their preparation, the type of compounds suitable for each matrix and preparation of standards. Typically the sample should be at a concentration of 1-100pmol/µl and the matrix at 1mg/ml. Ideally both the sample and matrix should be prepared in the same solvent which should evaporate easily at room temperature. The sample spots must be completely dry before the plate is put into the instrument to reduce the vacuum pump down time and the risk of arcing when the high voltage is applied to the extraction grid.

Instrument Operation

To load a plate into the instrument press the UNLOAD button on the instrument (1 in Fig. 1). When the flashing UNLOAD light changes to a steady LOAD light press the OPEN button (2 in Fig.1) to open the top cover (this may take a bit of effort as there is still a small residual vacuum in the sample chamber). Place the plate into the sample carrier platform ensuring that the corner indent is at the bottom right. Ensure the top of the sample plate is level with the carrier platform before closing the lid as if it is raised it may damage the extraction grid. Close the lid and press the LOAD button (3 in Fig. 1), this will start the load sequence which will take approximately 1-2 minutes. If the MassLynx software is not running, wait for the flashing LOAD light to change to a solid UNLOAD light before starting the software as this will cause hardware/software conflicts which will prevent the software working.

FIG.1

If you need to start the MassLynx programme, double click the MassLynx 4.1 icon on the desktop. This will take approximately 2 minutes to start up and will bring up the main window (Fig.2), if MassLynx is running then load your project as detailed below.

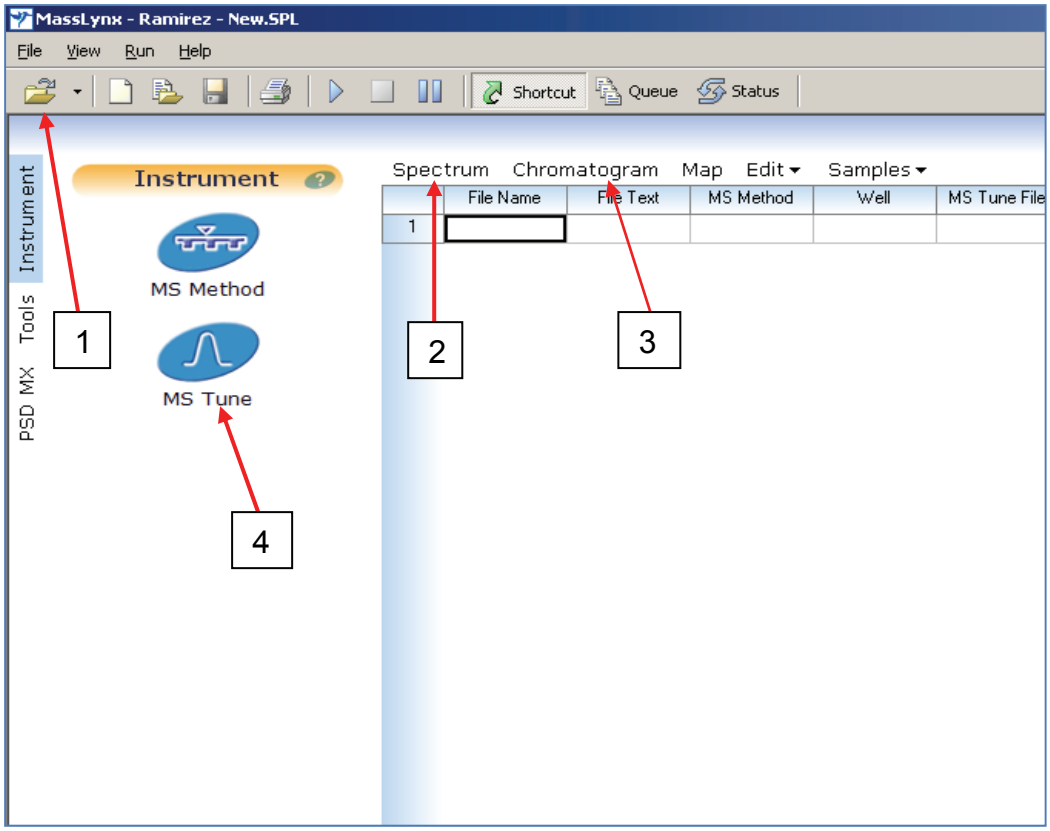


Fig.2

Load your project by clicking on the ‘Open File’ icon (1 in Fig.2) and selecting your project.

Open the Spectrum and Chromatogram windows by clicking on the links (2 & 3 in Fig.2).

Open the TUNE page by clicking on the MS TUNE link (4 in Fig.2). This will bring up the tune page which is used to set up and run the instrument (Fig. 3).

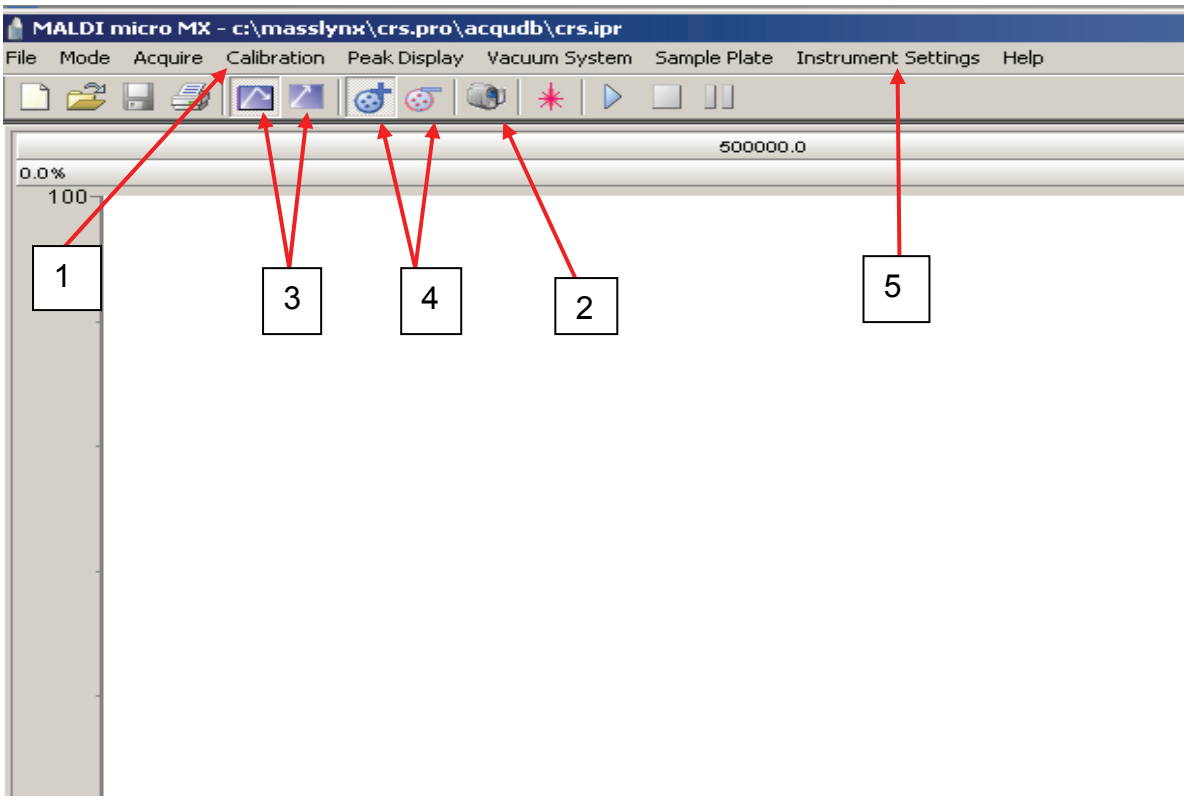
Click on the menu option ‘CALIBRATION’ (1 in Fig.3) and select ‘CALIBRATION ROUTINE’. This opens a calibration window (Fig 4). Click on FILE (1 in Fig 4), check that it is looking in your project and open the calibration file for your run. This will usually be called ‘pepmix pos.cal’ or ‘bsa lin.cal’ unless you are using a calibration file specific for your samples. Close the calibration routine window.

Click on the CAMERA icon (2 in Fig 3) to turn on the sample imaging camera.

Select which mode you require by clicking either the LINEAR or REFLECTION icon (3 in Fig. 3).

Select which ionisation type you require by clicking on the + or – icon (4 in Fig.3).

Select the ‘INSTRUMENT SETTINGS’ menu (5 in Fig.3) to open the settings window (Fig. 5).


MALDI micro MX - c:\masslynx\crs.pro\acqudb\crs.ipr
File Mode Acquire Calibration Peak Display Vacuum System Sample Plate Instrument Settings Help
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0.0%
100
1
3
4
2
5

Fig.3

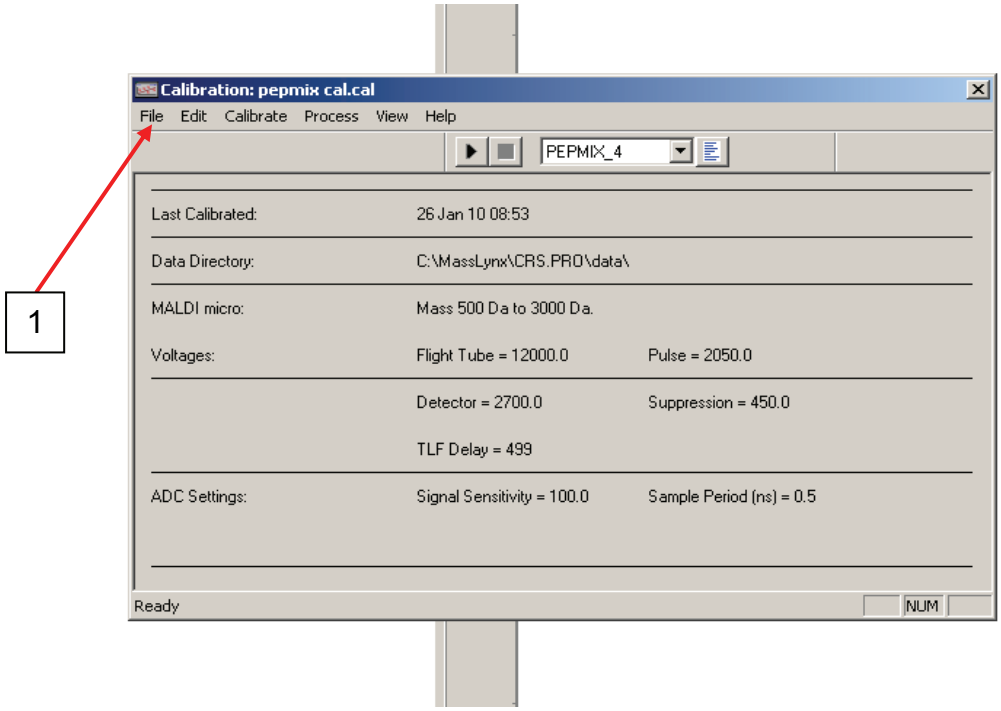
Calibration: pepmix cal.cal
File Edit Calibrate Process View Help
PEPMIX_4
Last Calibrated: 26 Jan 10 08:53
Data Directory: C:\MassLynx\CRS.PRO\data\
MALDI micro: Mass 500 Da to 3000 Da.
Voltages: Flight Tube = 12000.0 Pulse = 2050.0
Detector = 2700.0 Suppression = 450.0
TLF Delay = 499
ADC Settings: Signal Sensitivity = 100.0 Sample Period (ns) = 0.5
Ready
NUM
1

Fig 4

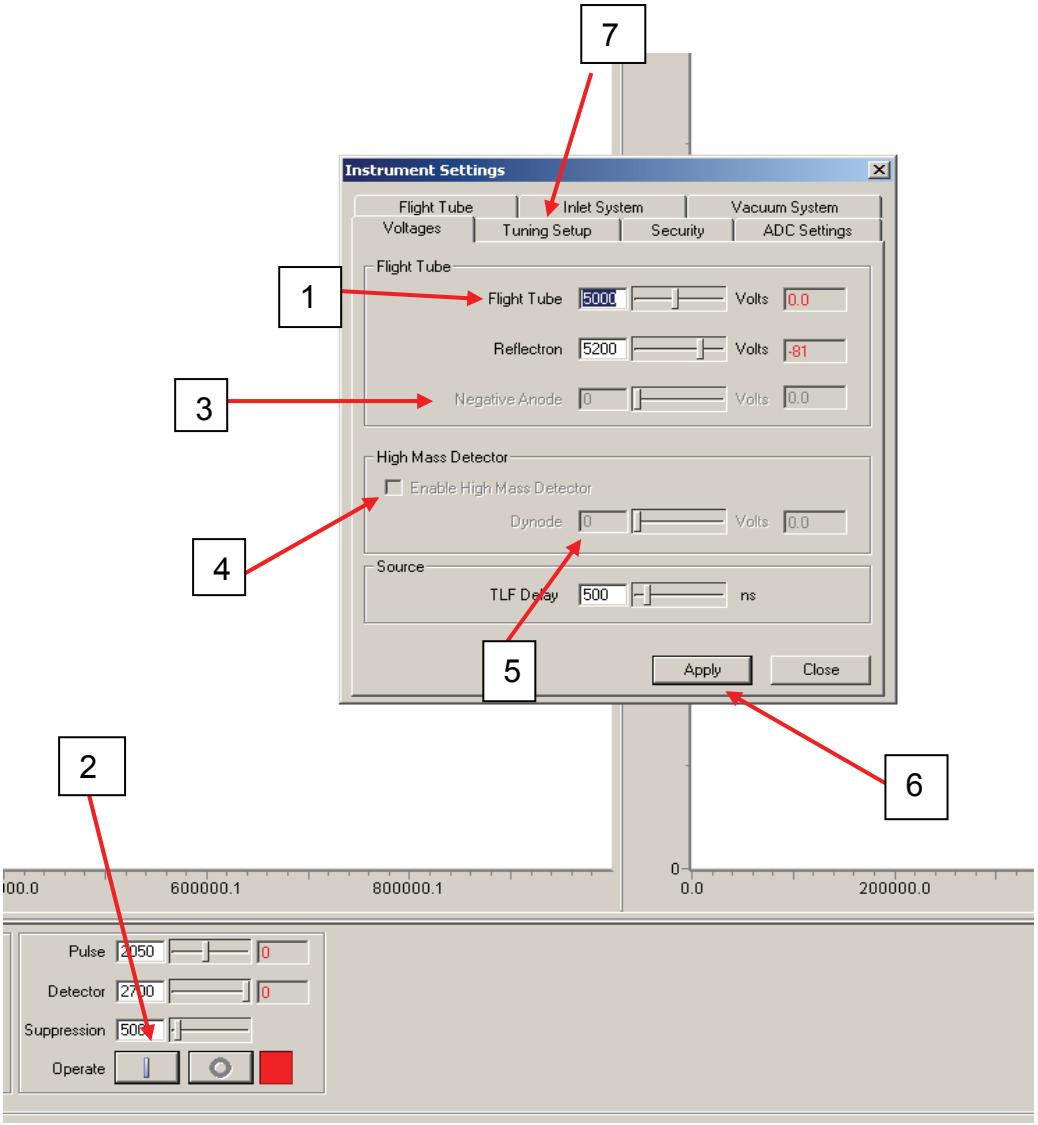

Fig. 5

Check that the ‘Flight Tube’ (1 in Fig. 5) is at 5000 volts, if not type this value in and press enter.

Click on ‘OPERATE’ (2 in Fig. 5). The RED square should turn GREEN and the red values in the Instrument Settings window will now show the voltages being applied.

Increase the FLIGHT TUBE voltage to its operating voltage of 12,000 volts in 2,000 volt increments, press ENTER after each input and check that the voltage increases.

The ‘REFLECTRON’ value should be at 5,200 volts and should not be changed.

If you have selected ‘NEGATIVE’ ionisation the ‘NEGATIVE ANODE’ slider (3 in Fig. 5) will be active. This should be raised from its starting voltage of 0 volts to its full operating voltage of 5,000 volts in 1,000 volt increments in the same manner as the flight tube voltage.

If you have selected ‘LINEAR’ mode the ‘REFLECTRON’ will be greyed out and the ‘HIGH MASS DETECTOR’ will be active. Click on the square ‘ENABLE HIGH MASS

DETECTOR' (4 in Fig. 5) and increase the 'DYNODE' voltage (5 in Fig. 5) from 0 volts to its operating voltage of 5,000 volts in 1,000 volt increments as above.

Click the 'APPLY' button (6 in Fig. 5) for these changes to be set. Click the 'TUNING SETUP' tab (7 in Fig. 5) to open the non-storage acquisition tuning page (Fig 6).

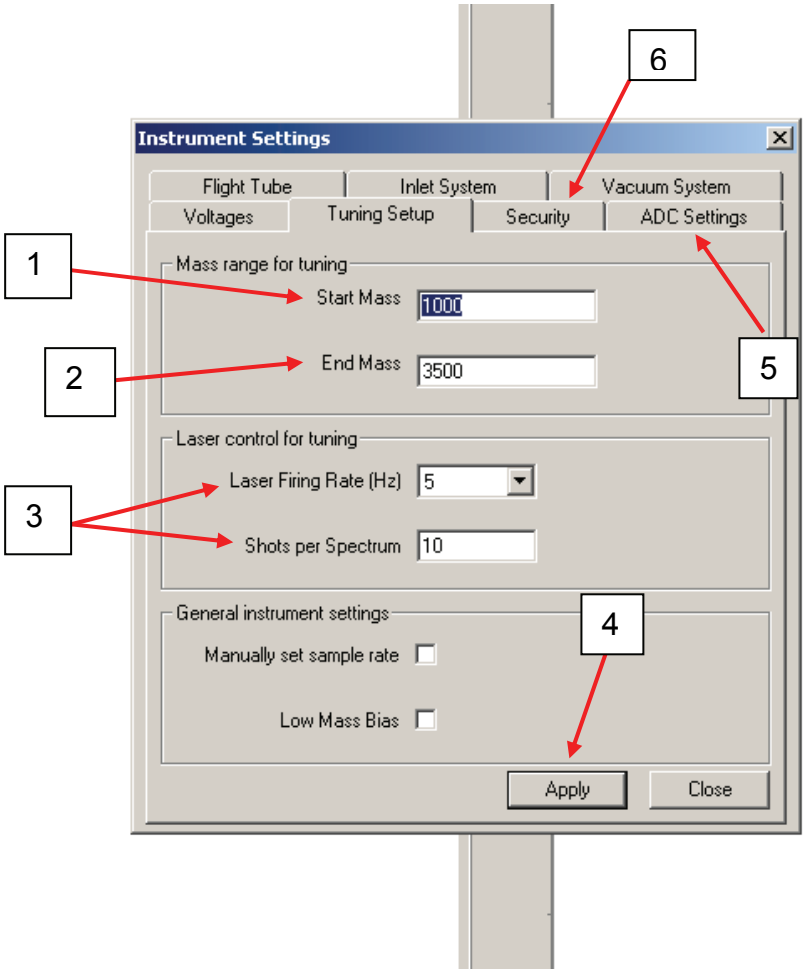

Fig. 6

Type in values for the 'START MASS' (1 in Fig. 6) and 'END MASS' (2 in Fig.6) to cover the mass range of your sample. This range will be used for displaying the spectra on the screen when not acquiring data and can be different to the range used to acquire data.

The 'LASER FIRING RATE' and 'SHOTS PER SPECTRUM' (3 in Fig. 6) should be set to 5 and 10 respectively.

Click on the 'APPLY' button (4 in Fig. 6) for the values to be set.

Click on the 'ADC SETTINGS' tab (5 in Fig. 6) to open this window (Fig. 7)

Initially this window will be greyed out. Check the 'SAMPLE PERIOD' (1 in Fig. 7).

For REFLECTRON this should be 0.5ns and for LINEAR 5ns. If the value is wrong click on the ‘SECURITY’ tab (6 in Fig. 6), type in the password and click ‘APPLY’ then return to the ‘ADC SETTINGS’ tab.

Click on the pull down menus for the ‘SAMPLE PERIOD’ and select the correct value for the mode being used.

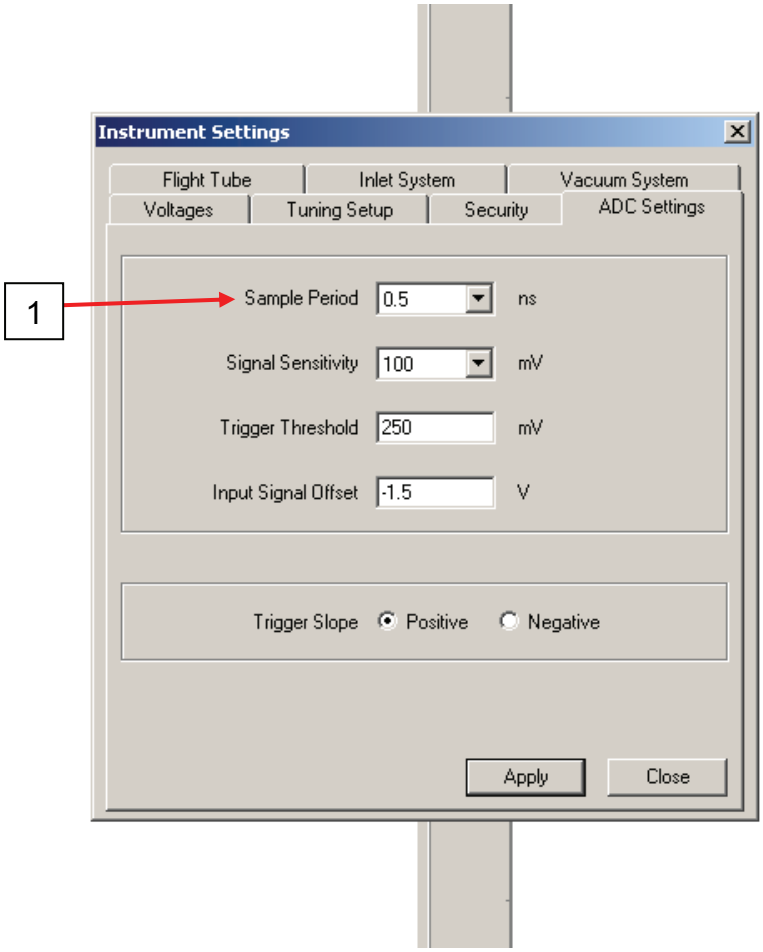

Fig. 7

Click on the ‘APPLY’ button then close the window.

The instrument is now ready to run samples.

Select the spot to analyse by typing the location into the ‘SAMPLE’ box (1 in Fig. 8).

The ‘PULSE’ (3 in Fig. 8), ‘DETECTOR’ (4 in Fig.8), ‘SUPPRESSION’ (5 in Fig. 8) and ‘LASER’ (2 in Fig. 8) are either set or adjusted as explained in the training.

The laser is steered using the cross hairs on the left (6 in Fig. 8) which are also seen on the camera image on the right (1 in Fig. 8a).

The right hand PEAK DISPLAY screen shows each individual laser shot and the left hand PEAK DISPLAY screen shows the summed spectrum (10 shots summed) (Fig. 9).

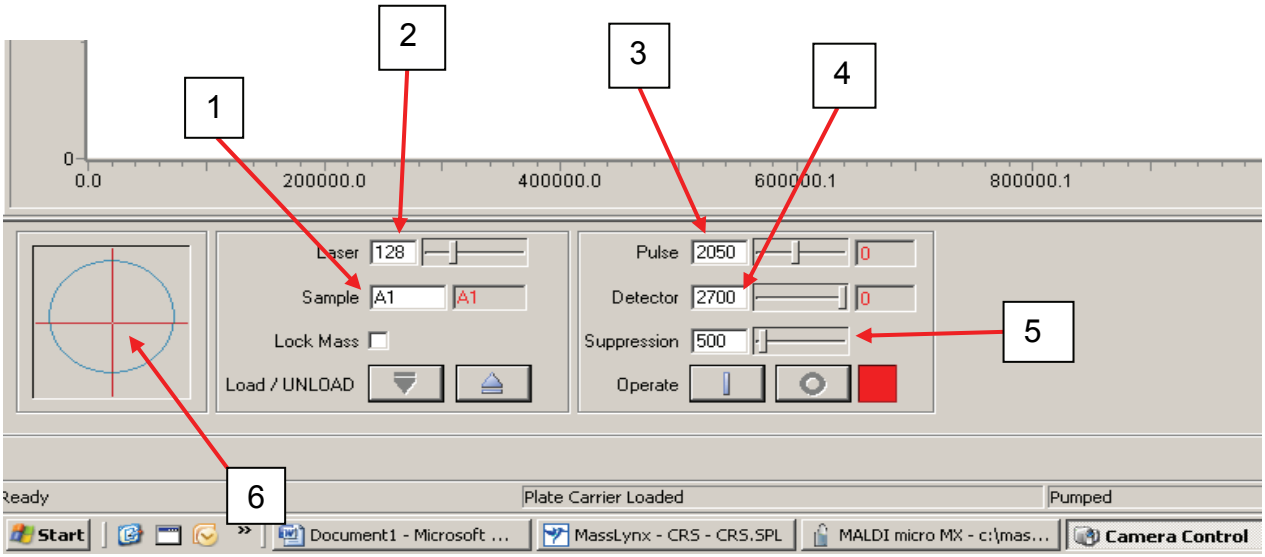

Fig 8

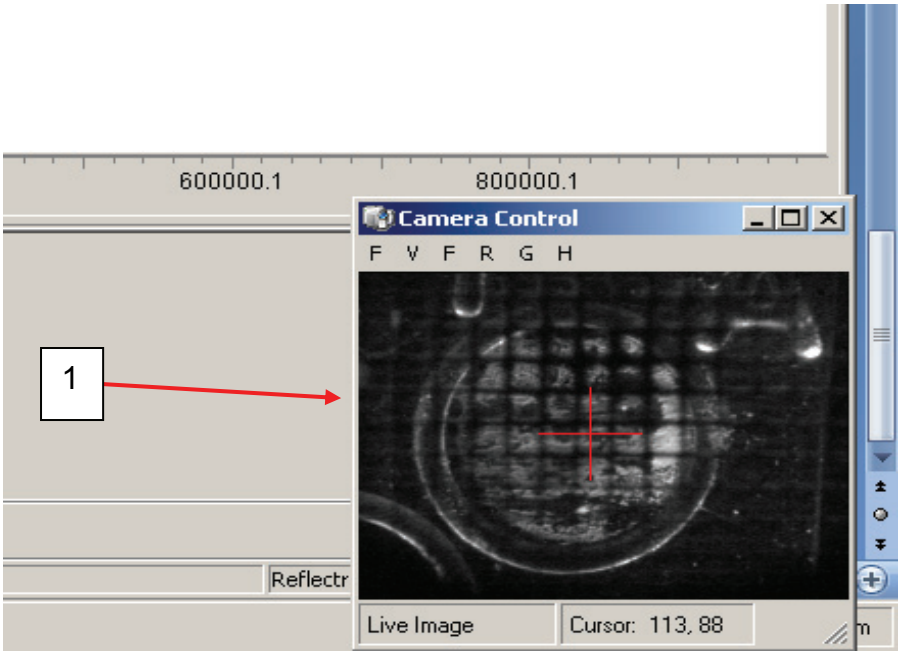

Fig 8a

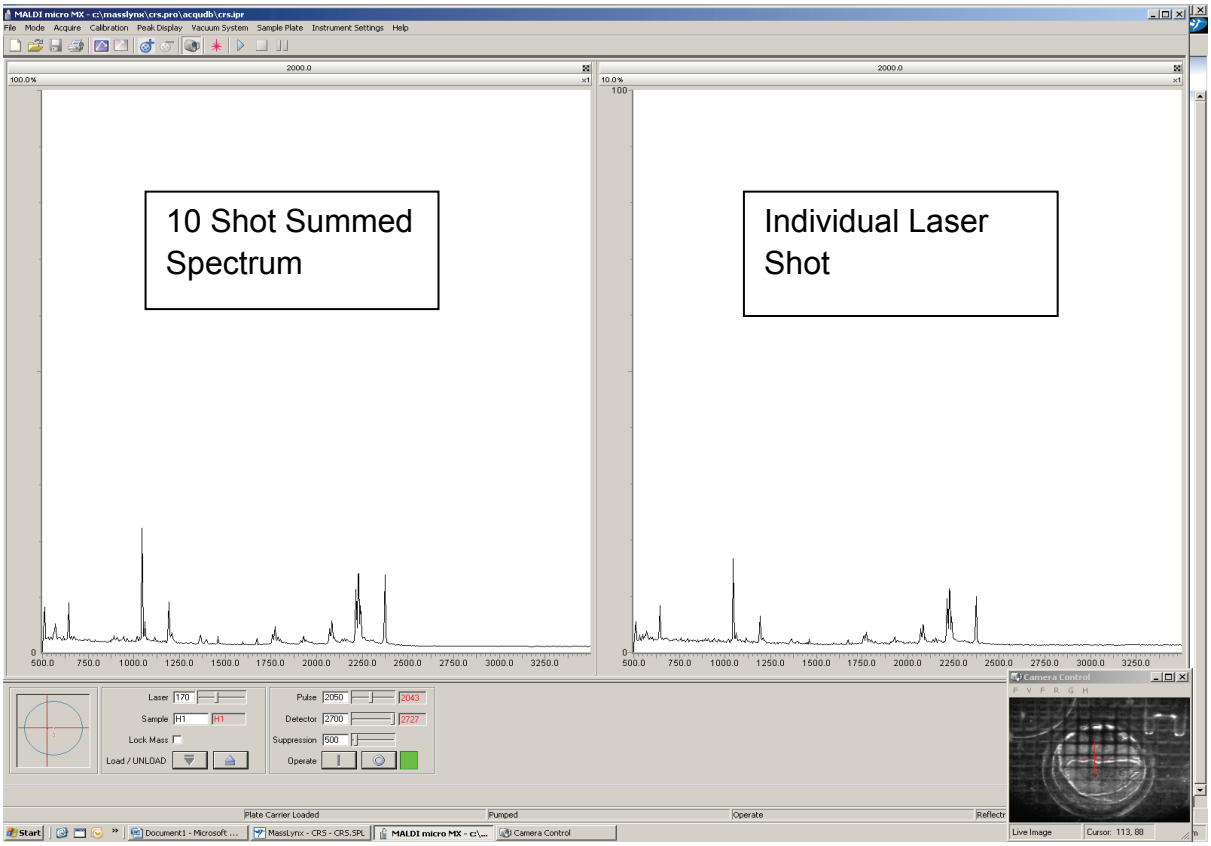
10 Shot Summed Spectrum
Individual Laser Shot

Fig. 9

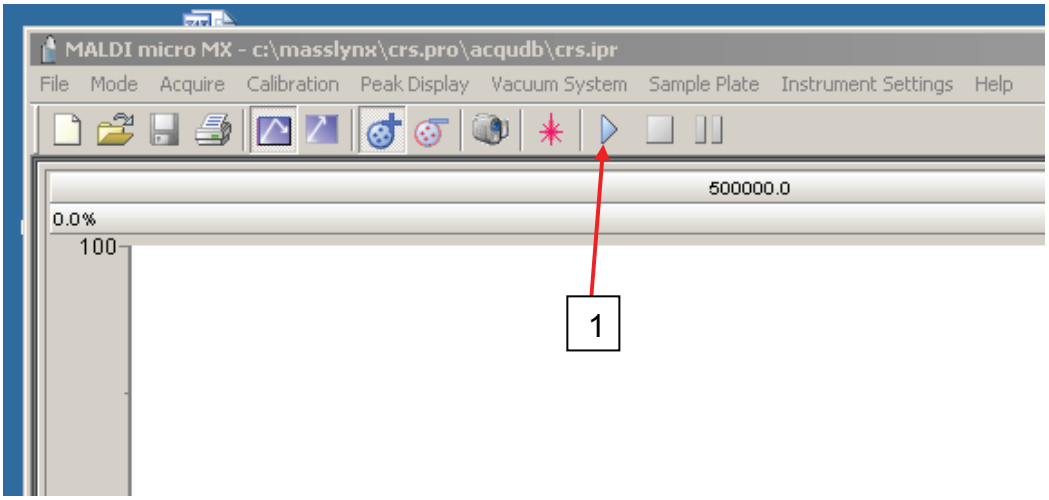
MALDI micro MX - c:\masslynx\crs.pro\acqudb\crs.ipr
File Mode Acquire Calibration Peak Display Vacuum System Sample Plate Instrument Settings Help
500000.0
0.0%
100
1

Fig. 10

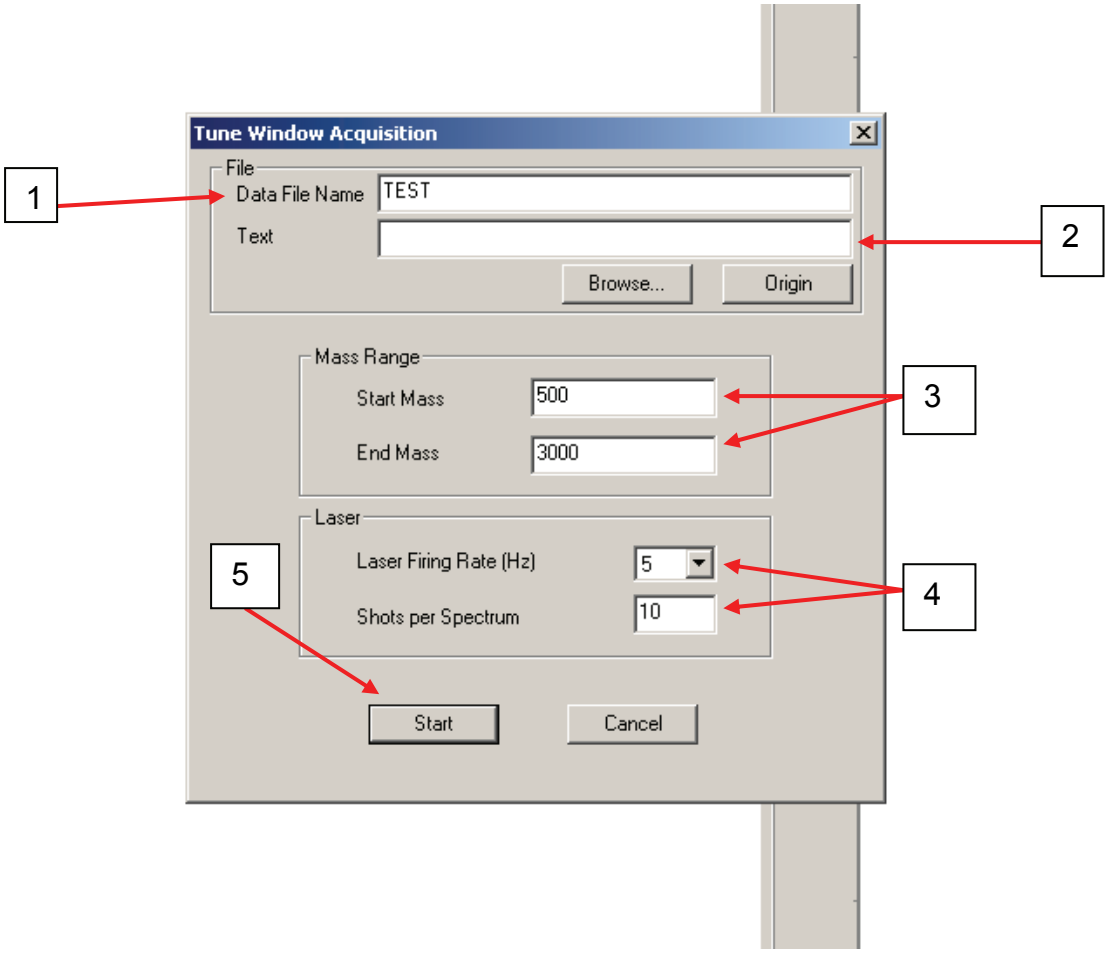

Fig 10a

To acquire data click on the ‘ACQUIRE’ icon (1 in Fig. 10) this will open a new window.

Click in the ‘DATA FILE NAME’ box (1 in Fig. 10a) and type in a name for the data file bearing in mind the restrictions on using some characters.

If you wish to add a text description to the results printout, type this into the ‘TEXT’ box (2 in Fig. 10a).

Type in the ‘START MASS’ and ‘END MASS’ values (3 in Fig. 10a) for the acquisition scan, this does not have to be the same as used for the non storage acquisition PEAK DISPLAY.

The ‘LASER FIRING RATE’ and SHOTS PER SPECTRUM’ (4 in Fig. 10a) should be 5 and 10 respectively as before.

To start the acquisition either press the ENTER key on the keyboard or the ‘START’ button (5 in Fig. 10a) in the acquisition window.

When you have finished running samples you should reset the ‘FLIGHT TUBE’ in the instruments settings window to 5000 volts, this can be done in 1 step and click the ‘APPLY’ button.

Close the CHROMATOGRAM & SPECTRUM windows, turn off the CAMERA using the icon (2 in Fig. 3), leave the instrument in ‘OPERATE’ and remove your plate from the instrument.

Ensure the instrument has started the sample plate load sequence and the LOAD light (3 in Fig. 1) is flashing before leaving.

REMEMBER TO TRANSFER YOUR DATA FILES FROM THE MALDI DATA SYSTEM TO ANOTHER STORAGE DEVICE FOR ARCHIVING.